Inhibition of *Mycobacterium abscessus* biofilms by *Methylobacterium* sp.

#P1443

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PURPOSE

❖ to study the effect of different forms of *Methylobacterium sp.:* live bacteria (LB), autoclaved (AB) and extract obtained after sonication (ES) on the percentage of covered surface, thickness and autofluorescence of *M. abscessus* biofilms using *confocal laser scanning microscopy* (CLSM)

MATERIALS AND METHODS

❖ *M. abscessus* DSM 44196 biofilm was developed using 2x4-well plates with an uncoated hydrophobic surface, incubated at 37º (80 rpm) for 96h.

❖ *Methylobacterium sp.* CECT 7805 was added in different forms (suspension of live bacteria (LB), autoclaved (AB) and an extract obtained after sonication (ES) at different times (24, 48 and 72 hours), leaving one well as a control (96 hours).
❖ The medium was replaced daily.
❖ The experiment was performed using the protocol previously described by Muñoz-Egea et al (BMC Microbiol 4 February 2015; 15:18). The statistical data were analyzed by pairwise comparisons using the nonparametric Mann-Whitney test with a level of statistical significance of $p<0.05$. All the experiments were performed in triplicate.

RESULTS

❖ The values of maximal inhibition of the percentage of covered surface and thickness of *M. abscessus* biofilm after exposure to all forms of *Methylobacterium sp.* were obtained at 72 hours, while the highest emission of autofluorescence was observed at 48 hours.
❖ After 72 hours of exposure, there were no statistical differences in thickness between LB and AB, but a statistical difference between LB and ES was detected, being higher the reduction obtained with LB. There were no differences between AB and ES.

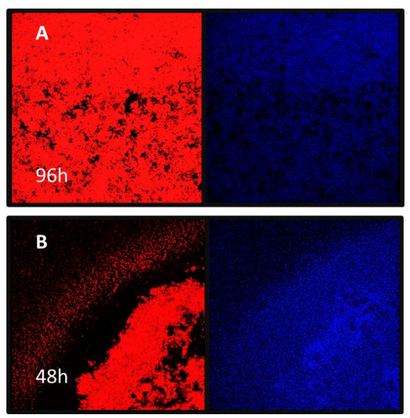


Figure 1. (A) 96h *M. abscessus* biofilm: Nile Red© stain (left), autofluorescence (right). (B) *M. abscessus* biofilm after expossure to LB for 48 hours: Nile Red© stain (left), autofluorescence (right).

RESULTS

❖ As for the percentage of covered surface, there was a significant difference at 72 hours between AB and ES, being higher the effect of AB in reducing this parameter. Similarly, it was shown a significant reduction in this parameter when comparing ES and LB, being higher the effect of LB. There were differences between AB and LB at 24 and 48 hours of exposure, being higher the effect produced by the live bacteria, although they were not statistically significant at 72 hours.
❖ Meanwhile, the percentage of autofluorescence was not significantly affected by the use of any form of Methylobacterium sp.

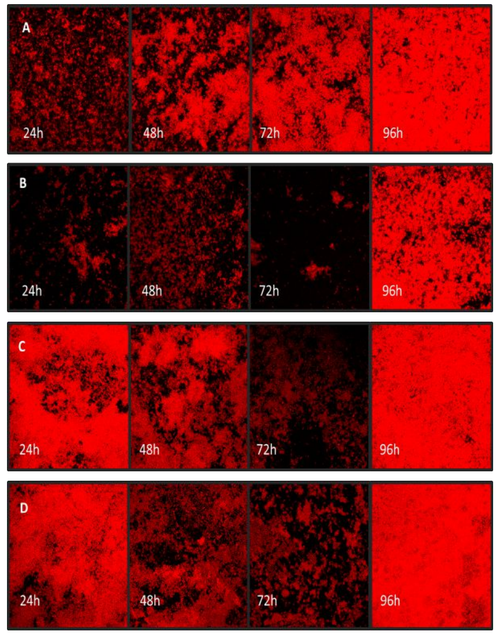
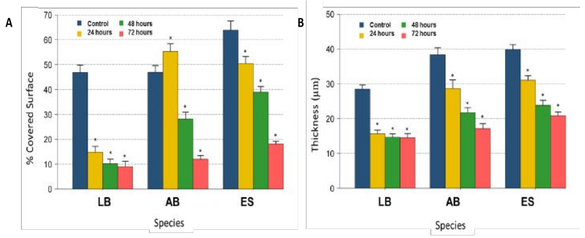


Figure 2. CLSM images of *Mycobacterium abscessus* biofilm formation at 24, 48, 72 and 96 hours (A). Inhibition assay of *M. abscessus* biofilm using living (B), autoclaved (C) and an extract of *Methylobacterium* (D). Images were taken after 24, 48 and 72 hours of exposure to *Methylobacterium*, using a 96h control of *M. abscessus* (Fig 1B, 1C). Nile Red© Stain.



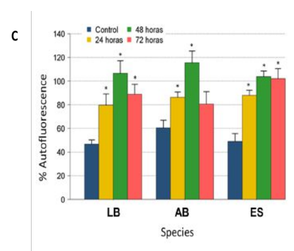


Figure 3. Effect of LB, AB and ES on (A) covered surface, (B) thickness and (C) autofuorescence of *M. abscessus* biofilm. Times: hours of exposure to *Methylobacterium* sp. (24, 48 and 72h) and 96h *M. abscessus* biofilm without *Methylobacterium* sp. as control. (*) Statistically significant different when compared to control ($p<0.05$).

CONCLUSIONS

❖ *Methylobacterium sp.* is able to inhibit *M. abscessus* biofilm formation, affecting both the thickness and the covered surface.
❖ *Methylobacterium sp.* alive is not necessary to inhibit a preformed biofilm of *M. abscessus* due to the addition of autoclaved *Methylobacterium sp.* and an extract of it showed an inhibition of *M. abscessus* biofilm. However, the inhibition of the covered surface and thickness of *M. abscessus* biofilm was significantly higher when it was exposed to the live *Methylobacterium sp*., especially after 24-48 hours.
❖ An increase in the autofluorescence emission by *M.abscessus* biofilms and extracellular fluorescence were observed after adding *Methylobacterium sp.*